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Impact of nitric oxide deficiency on blood pressure and glomerular hemodynamic adaptations to pregnancy in the rat

AIHUA DENG, KEVIN ENGELS, and CHRIS BAYLIS

Department of Physiology, West Virginia University, Morgantown, West Virginia, USA

Impact of nitric oxide deficiency on blood pressure and glomerular hemodynamic adaptations to pregnancy in the rat. Studies were conducted to investigate the impact of nitric oxide synthesis inhibition on blood pressure and glomerular hemodynamic adaptations to pregnancy in the rat. In normal pregnancy, urinary excretion of $NO_2 + NO_3$ (NO_X), reflecting increased nitric oxide (NO) production, progressively increased. Blockade of NO production in virgin and late pregnant Sprague-Dawley rats caused systemic hypertension, increased renal vascular resistance (RVR), reductions in RPF but GFR remained unchanged. In cortical nephrons, preglomerular and efferent arteriolar resistance (R_A and R_E) were elevated and glomerular capillary blood pressure (P_{GC}) increased markedly. Glomerular plasma flow (Q_A) and the glomerular capillary ultrafiltration coefficient, K_f, were reduced without change in single nephron glomerular filtration rate (SNGFR) because of the large elevation in P_{GC}. The pressor and glomerular hemodynamic responses to NO blockade were similar in virgins and pregnancy. Urinary NO_X excretion was markedly reduced in all groups with chronic NO blockade. Inhibition was incomplete in pregnancy, however, and a level of NO production that was adequate for normal BP and renal function in virgins, led to severe vasoconstriction in pregnancy. The present studies suggest that chronic NO deficiency leads to derangement of the hemodynamic adaptations of pregnancy.

Normal pregnancy is characterized by plasma volume expansion, vasodilation, falls in blood pressure (BP) and decreased sensitivity to vasopressor agents [1-4]. Renal vasodilation also occurs during midterm normal pregnancy, leading to increases in renal plasma flow (RPF) and glomerular filtration rate (GFR), which have returned to nonpregnant levels close to term [1, 2, 5]. The mechanism(s) of these physiologic adaptations are currently unknown although there is now evidence that the nitric oxide system is importantly involved. It is particularly critical to understand the mechanism(s) responsible for these adaptive changes in normal pregnancy, since in preeclampsia, a common pregnancy-induced disease in which hypertension develops, all these gestational adaptive responses are impaired [1, 6].

In recent years an endothelial control system has been discovered which plays a profound role in physiologic regulation of BP and renal function. In the nonpregnant rat, endogenously produced nitric oxide (NO) in vascular endothelial cells tonically contributes to control of BP and renal function [7, 8]. In the pregnant rodent the plasma level and urinary excretion of cGMP (the second messenger of NO), and $NO_2 + NO_3$ (NO_X; the stable oxidation products of NO) are increased [9], there is increased activity and expression of the endothelial NO synthase [10, 11], and recent evidence suggests that NO mediates the midterm gestational renal vasodilation [12]. Urinary cGMP excretion is increased in normal pregnant women although the impact of pregnancy on NO_X excretion is currently controversial [13, 14]. Thus, NO may mediate the physiologic systemic and renal hemodynamic adaptations of pregnancy.

Preeclampsia is clinically silent during the first half of pregnancy, and the symptoms of varying severity are manifest towards term [6]. There is evidence of general vascular endothelial damage in preeclampsia [15] and it is possible that a relative NO deficiency in pregnancy may result in the systemic manifestations of the disease. To investigate the consequences of NO deficiency in pregnancy, we determined the blood pressure and glomerular hemodynamic responses to chronic NO blockade in late pregnancy in the rat. We also measured 24-hour NO_x excretion as an index of overall NO production.

Methods

Studies were conducted on five groups of female Sprague-Dawley rats: normal virgins (NORV, $N = 5$), normal pregnants (NORP, $N = 6$), L-NAME-treated virgins, group 1 (NAMV1, $N = 5$), L-NAME-treated virgins, group 2 (NAMV2, $N = 5$), and L-NAME-treated pregnants (NAMP, $N = 6$). All rats were allowed free access to a standard rat pellet diet and tap water. Rats in the L-NAME-treated groups were placed on oral L-NAME in the drinking water for fourteen consecutive days immediately after receiving an i.v. bolus of L-NAME (10 mg/kg; a dose that produces the maximum, acute rise in BP [16]). The NAMP rats started L-NAME treatment on gestational day 4, and both NAMP and NAMV1 rats received L-NAME at a concentration of 100 mg L-NAME/liter drinking water, which was changed every other day. The amount of water intake was monitored, from which the daily L-NAME intake was calculated (Fig. 1). The L-NAME intake remained approximately constant in NAMV1 throughout the 14 day period (9.8 ± 0.2 mg/kg/24 hr) but increased progressively in NAMP (16.5 ± 0.5 mg/kg/24 hr) due to the increased water intake. In NAMV2 rats, each virgin female was paired to a NAMP rat and the concentration of L-NAME in the drinking water was progressively increased so that the 24-hour L-NAME intake was equal to that in the paired NAMP throughout the 14 day period and averaged 16.5 ± 0.5 mg/kg/min.

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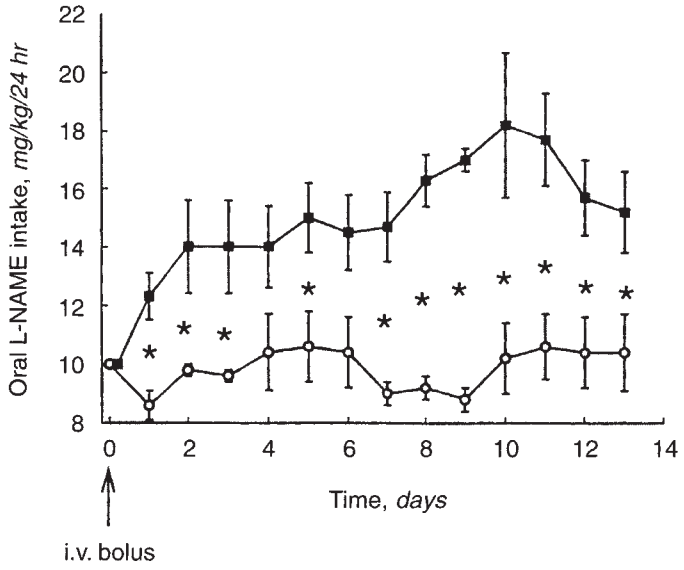

Fig. 1. *The daily oral intake of the NO synthesis inhibitor, nitro-L-arginine methylester (L-NAME) in a group of pregnant rats (NAMP; ■), given L-NAME in the drinking water from day 4 to day 18 of pregnancy.* A group of age-matched virgin rats (NAMV1; ○) also received L-NAME in the same concentration (100 mg/liter) over a 14 day period. *Denotes a difference in L-NAME intake between pregnant and virgin rats, due to the increased water intake in pregnancy.

Twenty-four hour urine collections were made before L-NAME, immediately after the i.v. bolus of L-NAME, day 6 after L-NAME treatment (midterm pregnancy, days 10 to 11), and day 13 of L-NAME treatment (late pregnancy, days 17 to 18), respectively, for measurement of urinary excretion of NO_X. In an additional group of four normal rats, 24 hour urines were collected in the virgin state, and at days 4 to 5, 10 to 11 and 17 to 18 of pregnancy; 24-hour food intake was also measured and the food analyzed for NO_X content. All 24-hour urine collections were frozen and later analyzed for NO_X content. On day 14 of L-NAME or drinking water, NORV, NORP, NAMV1 and NAMP rats were anesthetized and studied by glomerular micropuncture. NAMV2 rats were anesthetized and surgically prepared similarly to the other four groups and the arterial blood pressure (BP) was measured; however, these animals were too fragile to permit the lengthy, complete glomerular hemodynamic measurements.

Rats were anesthetized with Inactin (100 to 120 mg/kg, i.p.; Research Biochemicals Inc., Natick, MA, USA), placed on a temperature-regulated micropuncture table and rectal temperature maintained at 36° to 38°C. A tracheotomy was performed and polyethylene catheters (PE50) were inserted into the right femoral artery for blood sampling and measurement of arterial blood pressure (BP), into the right femoral vein for infusion of artificial plasma (2.5 g% bovine serum albumin, 2.5 g% bovine globulins in lactated Ringer solution) and into a jugular vein for infusion of tritiated inulin. BP was monitored by a Gould-Statham transducer connected to a direct-writing recorder (Gilson, Middleton, WI, USA). An i.v. infusion of artificial plasma was given at the rate of 1% body wt/hr for the first 45 minutes of surgery, thereafter, at 0.15% body wt/hr for the remainder of the experiment to maintain constant plasma volume. Isotonic NaCl solution containing ~100 μCi/ml tritiated-inulin was infused i.v. at a rate of 1 to 1.5 ml/hr during equilibration and throughout the experiment.

The left kidney was exposed through a midline and left subcostal incision, gently separated from the surrounding perirenal fat, supported on a flat Lucite plate and the kidney surface was illuminated with a fibre optic light source attached to a quartz glass light rod and slowly superfused with warm, 0.9% NaCl solution (34 to 36°C). The left ureter was catheterized with PE10 for collection of urine and a catheter (PE50) attached to a bent, cut 26G needle was inserted, retrograde, into the left renal vein for periodic sampling of renal venous blood. A 60-minute equilibration period was allowed after completion of surgery before any measurements were made.

Two exactly timed urine collections (25 to 30 min) were made directly into graduated glass tubes for determination of urine flow (V) and urinary inulin concentration. Blood samples (200 μl) were taken at the midpoint of urine collection periods from the femoral artery and renal vein for measurements of hematocrit (hct), plasma protein concentration (C_A) and plasma inulin concentration. Coincident with the urine collections, the following micropuncture measurements were made. Exactly timed (2 to 3 min) samples of tubule fluid were collected at random sites on the perfused kidney surface by puncture of six to eight surface proximal convoluted tubules, for determination of inulin content. Efferent arteriolar (post-glomerular) blood was collected by puncture of three to five superficial star vessels for determination of efferent arteriolar plasma protein concentration (C_E). Hydrostatic pressures were measured in surface efferent arterioles and proximal tubules using the servo-null micropressure measuring system (Model 4A; IPM, San Diego, CA, USA). P_{GC} was measured indirectly: wax blocks were inserted into five to seven mid-proximal surface segments to completely obstruct fluid flow. The hydrostatic pressure in the tubule proximal to the block rises until it reaches the "stop-flow" pressure (P_{SF}), when filtration stopped. P_{SF} was measured at the earliest proximal segment.

The activities of tritiated inulin were measured in aliquots of arterial and renal venous plasma (5 μl), urine (1 μl) and the entire tubular fluid sample, which allowed calculations of SNGFR, GFR, RVR and RPF. Protein concentrations (C) in femoral (C_A) and efferent arteriolar (C_E) plasma were measured using a microadaptation of the Lowry method [17] and the colloid osmotic pressures were calculated [18]. Together, these measurements allow calculation of SNGFR, single nephron filtration fraction (SNFF), Q_A, R_A, R_E and K_f [18]. Since the values of SNGFR were obtained from proximal collections, these may slightly overestimate the true SNGFR, because of the interuption of flow to the macula densa. Urinary NO_X concentration was measured by a reduction of urinary NO_3 to NO_2 with the nitrate reductase enzyme and the NO_2 generated from NO_3 by the nitrate reductase enzyme, and any NO_2 in the urine was detected and quantitated by the Griess reaction [19]. Briefly, 125 μl urine samples (diluted 3 to 4×) were incubated with 100 μl buffer (1.0 M HEPES, 2.4 mM ammonium formate, pH 7.2) and 25 μl nitrate reductase enzyme (100 mg/ml), for one hour at 37°C in a shaking water bath. The tubes were spun at 2000 g for 10 minutes and the 100 μl of the supernatant was mixed with 150 μl Griess reagent in 96 well plates and read at 543 nm in an ELISA plate reader. NO_2 standards were run in the range of 5 to 500 mM as well as a 100 mM NO_3 standard, to test for complete reduction by the enzyme. The NO_X content of the rat food was measured in aliquots of solutions of

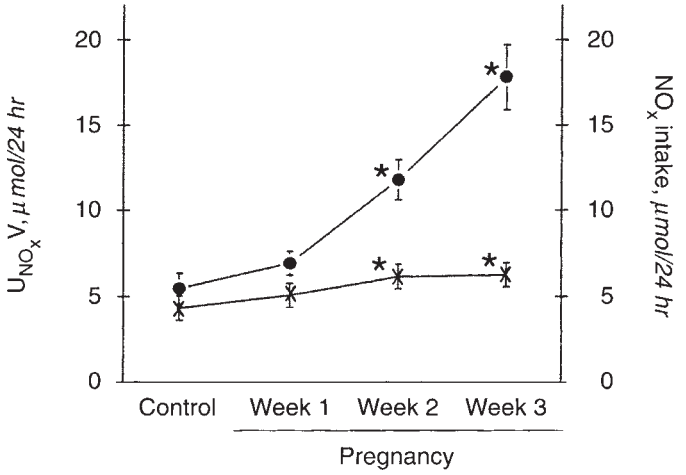

Fig. 2. *The 24-hour urinary excretion of nitrate + nitrate ($U_{NOX}V$; ●) the stable oxidation products of nitric oxide (NO), in a group of normal rats, before and early (week 1), mid (week 2) and late (week 3) in pregnancy.* The NO_X intake in the food (**X**) is also shown. *Denotes a difference versus the control, pre-pregnancy value.

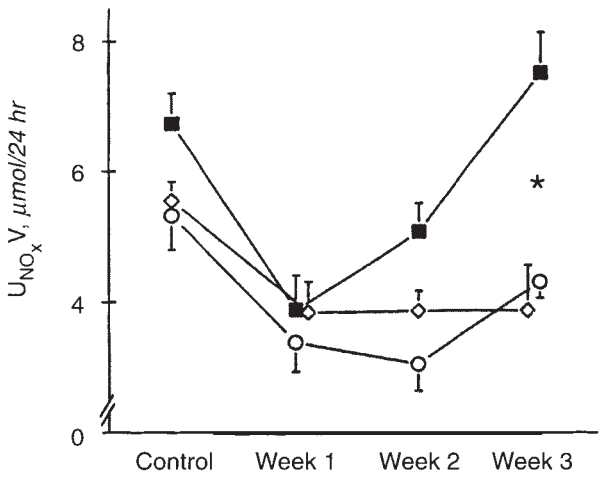

Fig. 3. *The 24-hour urinary nitrate + nitrite excretion ($U_{NOX}V$) in the control, baseline state and in a group of pregnant rats (NAMP; ■; BP = 154 ± 5) receiving L-NAME from day 4 to day 18 of pregnancy in an average dose of 16.5 ± 0.5 mg/kg/24 hr.* Data are also shown for 2 groups of virgin rats given L-NAME over a 14 day time course, NAMV1 (○; BP = 145 ± 7) received an average dose of 9.8 ± 0.2 mg/kg/24 hr and NAMV2 (◇; BP = 186 ± 6) received an average dose of 16.5 ± 0.5 mg/kg/24 hr. *Denotes a difference ($P < 0.01$) in $U_{NOX}V$ at week 3 between pregnant rats and both groups of virgins.

food dissolved in distilled water (0.05 to 0.3 g/ml) and was found to average 263 nmol NO_X/g of food.

The statistical comparison of all renal functional data were made by unpaired *t*-test comparing virgin and late pregnancy within either control or L-NAME-treated groups and by ANOVA for certain preplanned comparisons between groups. Statistics on NAME intake and 24-hour urinary excretion of NO_X within groups were done by repeated measures ANOVA with univariate tests of hypotheses for within subjects effects. Comparisons on these variables between groups were by repeated measures ANOVA with analysis of variance of contrast variables. Statistical significance is assumed where $P < 0.05$.

Results

As shown in Figure 2, 24-hour urinary NO_X excretion increased progressively during pregnancy so that by close to term, $U_{NOX}V$ had increased three to four times above the virgin value. As also shown, dietary NO_X intake also increased because of increased food consumption, but the increased intake could only account for a small fraction of the increased urinary output, suggesting that increased NO production in pregnancy must account for the balance. Figure 3 gives the 24-hour $U_{NOX}V$ values for NAMP, NAMV1 and NAMV2 rats before and during pregnancy. The mean BP for each group, obtained in the terminal study, is given on the far right. In NAMP, NAME lowered $U_{NOX}V$ to below the nonpregnant baseline value both in early and mid-pregnancy; however, by late pregnancy, $U_{NOX}V$ rose back to the baseline (virgin) unblocked value despite a continued high (increasing) NAME intake (Fig. 1), which was on average 16.5 ± 0.5 mg/kg/24 hr. Nevertheless, the BP in these late pregnant rats was high (155 ± 4 mm Hg). In virgins on a matched NAME intake (NAMV2), a maintained and greater suppression of NO production (vs. NAMP) was seen and BP was higher (186 ± 6 mm Hg) versus pregnant rats (Fig. 3). In NAMV1 rats, which received the lower dose of L-NAME (an average of 9.8 ± 0.2 mg/kg/24 hr), NO blockade (as assessed by reduced 24 hr $U_{NOX}V$) was also maintained for the 14 day period of L-NAME administration and was similar to the NAMV2 group. Of note, $U_{NOX}V$ was lower in NAMV1 rats than in NAMP, despite the fact that NAMV1 rats had received a lower dose of L-NAME. BP was elevated in both groups of virgins but by less in NAMV1 versus NAMV2 rats ($P < 0.01$), despite a similarity of 24-hour $U_{NOX}V$ profiles (Fig. 3).

Data are summarized for whole kidney and single nephron function in NORV, NORP, NAMV1 and NAMP groups in Table 1.

Normal pregnant versus normal virgins

Normal late pregnant rats (NORP) exhibited significant decreases in hct compared to normal virgins (NORV), suggesting plasma volume expansion, and a late gestational reduction in BP, suggesting a decline in total peripheral vascular resistance (TPR). There was no difference in renal or glomerular hemodynamics between late pregnant rats and normal virgins. This is consistent with previous findings by us and others that the increased glomerular filtration rate (GFR), renal plasma flow (RPF), single nephron glomerular filtration rate (SNGFR) and glomerular plasma flow (Q_A) and decreased total renal vascular resistance (RVR) seen at midterm pregnancy, return towards nonpregnant values close to term [5].

NAME treated versus normal virgins

In virgin rats, chronic systemic NO blockade caused increased hct (presumably secondary to volume contraction), systemic hypertension and increased RVR that led to pronounced reductions in RPF. GFR was unchanged and thus filtration fraction (FF) increased (Table 1). In the cortical nephron population, both R_A and R_E were elevated with chronic NO blockade. Because of the increase in R_E and BP and despite the rise in R_A, P_{GC} rose markedly. Q_A was significantly reduced due to the increases in segmental arteriolar resistances and there was no change in SNGFR because of the large elevation in hydrostatic pressure

Table 1. Summary of whole kidney and single nephron hemodynamic changes in normal control (NOR) and L-NAME (NAM) treated late pregnant (P) and virgin (V) rats

	Hct	BP	GFR	RPF	FF	RVR	SNGFR	Q_A	Π_a	Π_E	P_{GC}	R_A	R_E	K_f
	%	mm Hg	ml/min			mm Hg/(ml/min)	nl/min		mm Hg			dyn · s · cm^{-5} × 10^{10}		nl/s/(mm Hg)
NORP	36	88	0.79	2.72	0.30	20	35	127	17	28	47	1.8	1.6	0.044
	±1	±3	±0.06	±0.17	±0.01	±1	±2	±11	±0.5	±1	±1	±0.3	±0.3	±0.004
NORV	41	101	0.77	2.67	0.31	24	33	125	17	28	49	2.0	1.6	0.040
	±1	±3	±0.07	±0.24	±0.03	±4	±3	±11	±0.3	±1	±1	±0.2	±0.2	±0.003
NAMV	44	145	0.72	1.61	0.46	58	40	91	19	47	75	3.5	4.1	0.021
	±1	±7	±0.10	±0.24	±0.01	±14	±2	±12	±0.9	±2	±2	±0.2	±0.3	±0.002
NAMP	38	154	0.73	1.71	0.42	58	38	95	19	44	77	4.4	5.3	0.018
	±2	±5	±0.07	±0.17	±0.02	±7	±4	±12	±0.6	±2	±2	±0.8	±0.9	±0.002
P: NORP vs. NORV														
	<0.01	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P: NORV vs. NAMV														
	<0.01	<0.01	NS	<0.02	<0.01	<0.01	NS	<0.05	NS	<0.01	<0.01	<0.05	<0.01	<0.01
P: NAMV vs. NAMP														
	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P: NORP vs. NAMP														
	<0.01	<0.01	NS	<0.02	<0.01	<0.01	NS	<0.05	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01

All data are mean ± SE. Statistics are done by ANOVA. Groups are: NAMV1, NAME-treated virgin (9.8 ± 0.2 mg/kg/24 hr); NAMP, NAME-treated late pregnant (16.5 ± 0.5 mg/kg/24 hr); NORV, normal virgin; NORP, normal late pregnancy.

gradient ($P_{GC} - P_T$), and thus, single nephron filtration fraction, SNFF increased (not shown). π_A did not change and π_E increased significantly. The glomerular capillary ultrafiltration coefficient, K_f, was reduced by ~50%.

NAME treated pregnant versus NAME treated virgins

NO blockade prevented the normal gestational late fall in BP, and in fact produced systemic hypertension in late pregnant (NAMP) rats. Both BP and P_{GC} were similarly and markedly elevated in NAMP and NAMV1 rats, despite the fact that NO production is higher in NAMP. At the whole kidney and single nephron level a similar pattern of vasoconstriction, reduced plasma flow and K_f and unchanged filtration rate was seen in both groups of L-NAME treated rats. The only hemodynamic difference between NAMP and NAMV was a significant decrease in hct in NAMP, reflecting persistent plasma volume expansion (Table 1).

NAME treated versus normal pregnant rats

Compared with normal pregnancy (NORP), chronic L-NAME treated pregnant (NAMP) rats developed severe systemic hypertension. At the whole kidney level an increase in FF was the result of a reduction in RPF due to profound increases in RVR with unchanged GFR. At the single nephron level, π_A did not change and π_E increased. There were large rises in segmental arteriolar resistances, R_A and R_E. As with NAMV, the rise in R_E, together with increased BP resulted in glomerular capillary hypertension, despite the increase in R_A. Q_A was reduced with unchanged SNGFR leading to a rise in SNFF. With chronic NO blockade in pregnant rats K_f was reduced by approximately 50% compared with normal pregnant rats. Overall, both virgin and late pregnant rats responded similarly to chronic systemic NO blockade.

Discussion

The present studies confirm earlier work by Conrad and colleagues [9], that during normal pregnancy in rats, the 24-hour urinary excretion of NO_X increases progressively and reaches a maximum by late pregnancy. The amount of 24 hour $U_{NOX}V$ is influenced by NO_X intake (in the diet), absorption from the GI tract, renal handling of NO_X, as well as *de novo* NO synthesis [20]. Based on metabolic studies, it seems that balance is rapidly restored (within 24 to 48 hr) when NO_X intake is varied and the majority of NO_X excretion is via the kidney [20]. Therefore, when corrected for NO_X intake, 24-hour $U_{NOX}V$ reflects total (peripheral and renal) NO production and as shown by us, the gestational rise in $U_{NOX}V$ greatly exceeds the dietary NO_X intake, demonstrating a net increase in NO production. In pregnancy the maximum rise in $U_{NOX}V$ excretion occurs late in pregnancy and is correlated with the fall in BP due to the reduction in peripheral vascular resistance. At this time the RVR is not different from the nonpregnant value, and thus we anticipate that renal NO production is unlikely to be high. Therefore we interpret the increased urinary NO_X excretion of late pregnancy to reflect increased NO production in other vascular beds that causes the late gestational decline in peripheral vascular resistance. It is likely that the increase in urinary NO_X reflects NO generated from vascular endothelium, peripheral and central neuronal sites as well as elsewhere in the body, and may thus be reflecting nonvasodilatory as well as vasodilatory NO. In the studies by Conrad et al, in addition to 24 hour $U_{NOX}V$, increases in plasma NO_X concentration were also observed during gestation that were closely correlated with increases in cGMP, the second messenger of NO [9]. Increased cGMP has also been reported in earlier studies in both normal human and rat pregnancies [13, 21], and in the rat the metabolic clearance rate of cGMP was not altered. Therefore, the increase in urinary and plasma cGMP reflected increased tissue cGMP production [21].

In vivo studies suggest that NO is responsible for the pregnancy associated refractoriness to the pressor action of administered vasoconstrictors [4], although *in vitro* studies have been more variable and show substantial differences in the role of NO in various regional vascular beds [22-25]. Increased eNOS has recently been reported in the aorta of the pregnant rat [11], and

in guinea pigs the activity of calcium-dependent (constitutive) NO synthase (NOS) is increased in uterine artery, kidney, heart as well as other tissues in both early and late pregnancies [10, 26]. Increased expression of mRNA levels for both constitutive NOS isoforms (endothelial and neuronal) have been observed in a variety of locations in late pregnant guinea pig, and estrogen may provide the primary stimulus [10]; however, since estrogen levels are low in rats until late in pregnancy [27] the precise role of estrogens remains to be determined.

In addition to a general increase in vascular NO production, which contributes to the gestational vascular refractoriness and fall in BP, increased NO production apparently plays a role in the renal vasodilation of pregnancy. It is clear that NO is an important, physiologic renal vasodilator [8]. Recent work in the conscious pregnant rat suggests that the midterm renal vasodilation is due to increased NO production [12]. Finally, chronic NO blockade during pregnancy leads to suppression of the normal peripheral and renal vasodilation [28] and produces a pattern that resembles the symptoms of preeclampsia. Therefore, increased NO production may be responsible for the peripheral and renal vasodilation in the normal pregnant rat.

In the present study we have further investigated the effect of chronic NO blockade in pregnancy in the rat. We examined the effect of chronic L-NAME on 24-hour urinary NO_X excretion throughout pregnancy, and on BP and glomerular hemodynamics close to term. Since renal and hypertensive complications of preeclampsia manifest late in human pregnancy, we chose to make our functional observations in close to term rats. These studies show that NO synthesis is more difficult to suppress in pregnancy, which presumably reflects enhanced synthetic capacity. Even with incomplete suppression of NO synthesis, prevention of the normal large gestational rise in NO production results in hypertensive pregnancy. In fact, a level of NO production that is appropriate for control of vascular tone in normotensive (non-NO blocked) virgins is inadequate in the L-NAME-treated pregnant rat. Therefore, BP and RVR increase with the development of glomerular capillary hypertension. These high values of P_{GC}, if sustained, will lead to glomerular structural damage [5]. Of particular note, NO production must be increased in late pregnancy in order to accomodate the altered hemodynamic status and permit the late fall in BP. This increased NO production in normal pregnancy presumably acts by maintaining a new balance between vasodilator and vasoconstrictor influences, thus accommodating the expanded plasma volume.

It is likely that the systemic and renal hemodynamic responses to chronic NO inhibition in nonpregnant states as well as in pregnancy are the result of an imbalance between vasodilatory and vasoconstrictor systems. In normal pregnancy the plasma level of angiotension II (Ang II) is elevated and systemic vascular response to administered Ang II is blunted [1, 3]. Studies in the rat have suggested that pregnancy-induced refractoriness to Ang II as well as other vasopressor agents, AVP and NE, is due to NO since the refractoriness can be reversed by acute inhibition of NO synthesis [4]. The relationship between NO and Ang II in control of RVR depends on the level of endogenous intrarenal Ang II, and when Ang II levels are elevated, renal hemodynamics are largely mediated by the balance between NO and Ang II [8]. Therefore, chronic NO synthesis inhibition will amplify actions of Ang II when the Ang II synthesis is increased, as occurs in pregnancy. Other vasopressor systems may also be potentiated during states of NO deficiency.

Chronic partial NO blockade in male rats causes hypertension, renal vasoconstriction, glomerular hypertension, falls in K_f and eventual glomerular injury after approximately two months [29]. In the present study, two weeks of NO blockade in virgin females causes hypertension, marked renal vasoconstriction with increases in both R_A and R_E, severe glomerular hypertension and falls in K_f, probably secondary to mesangial cell contraction [8]. A similar pattern was seen in chronically NO blocked late pregnant rats. In fact, the only significant hemodynamic difference between virgin and pregnant chronically NO blocked animals was the lower hct in NAMP that was indicative of some persistent plasma volume expansion. Of importance, the normal late gestational fall in systemic BP was suppressed such that BP was high in NAMP and nonsignificantly greater than NAMV. It should be emphasized that similar hypertensive and renal vasoconstrictor responses occurred in pregnant and virgin rats, despite higher levels of residual NO synthesis in pregnant rats. Presumably, if equivalent levels of NO synthesis inhibition had been achieved in pregnant and virgin rats given NAME, the pressor and renal vasoconstrictor response in pregnancy would be exaggerated.

In this and in earlier studies we observed that in normal pregnancy, the RVR is increasing back towards the nonpregnant value, close to term. Thus, GFR, SNGFR, RPF, Q_A, R_A, R_E and P_{GC} are similar in late pregnant and virgin rats, despite the large volume expansion (reflected by low hct), peripheral vasodilation and a fall in BP [5]. In comparing the NO blocked (NAMP) and normal (NORP) pregnant rats it is evident that systemic BP, RVR, R_A, R_E and P_{GC} are much higher, while RPF, Q_A and K_f are much lower in NO-blocked versus normal pregnant rats. Despite the marked renal vasoconstriction and low K_f of NO deficiency, the glomerular hypertension is more than sufficient to prevent declines in GFR or SNGFR.

In this study, we have shown that the normal vasodilatory effect of pregnancy is lost due to NO blockade. This contrasts with other hypertensive states where late pregnancy is uniformly antihypertensive in the spontaneously hypertensive rat, DOCA/salt hypertension, Goldblatt hypertension, severe renal ablation and adrenal regeneration [30-32]. We have also found that in rats with 5/6 renal ablation, the kidney as well as the periphery vasodilates close to term [32]. Thus, chronic NO blockade-induced hypertension is unique in preventing the midterm renal and later peripheral vasodilation response to pregnancy, and suggests a causal role for NO in mediating the gestational vasodilation. Recent preliminary observations suggest that L-arginine supplementation reverses the hypertension, proteinuria and growth retardation seen with chronic L-NAME administration to pregnant rats, further reinforcing the notion that the adverse changes with L-NAME result from NO synthesis inhibition [33].

At present, there is no consensus from clinical studies on the impact of normal or preeclamptic pregnancy on NO production, as measured by NO_X and certain problems with the clinical assessment of NO activity, by NO_X determination, may be responsible [14]. A relative arginine deficiency develops in normal pregnant women, perhaps reflecting increased NO synthesis [34-36]. The circulating endogenous NOS inhibitor asymmetric dimethyl arginine (ADMA) falls in normal pregnancy and increases in preeclampsia [37], providing one mechanism by which NO

production would be reduced. The few studies on vascular reactivity in maternal resistance vessels are conflicting. Although no changes are seen in endothelium dependent relaxation of small arteries from normal pregnant or preeclamptic women studied *in vitro* [38, 39], an exaggerated vasoconstriction to NO synthesis inhibition occurs *in vivo* in the hand circulation of normal pregnant versus nonpregnant women, suggesting increased tonic NO release [40]. Increased 24-hour urinary excretions of cGMP seen in normal pregnancy are reduced in preeclampsia [13, 14, 41].

To summarize, the adverse interactions between pregnancy and hypertension induced by NO blockade in rats in the present and a previous study [28] include systemic hypertension sustained throughout the pregnancy, prevention of the normal midterm gestational renal vasodilation, high values of RVR and P_{GC} at term leading, not surprisingly, to proteinuria as well as poor fetal and maternal outcomes. These are similar to the symptoms seen in preeclamptic women. In late pregnancy this vasoconstricted state with chronic NAME exists despite absolute levels of NO production that are equivalent to normal (unblocked) NO levels seen in the virgin. Therefore, pregnancy requires large increases in NO production to accomodate the increased level of vasoconstrictor influences and expanded plasma volume.

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Reprint requests to Chris Baylis, Ph.D., Department of Physiology, P.O. Box 9229, West Virginia University, Morgantown, West Virginia 26506-9229, USA. email: baylis@wvnvms.wvnet.edu

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